

In Vitro Liberation of Indomethacin from Chitosan Gels Containing Microemulsion in Different Dissolution Mediums

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ABSTRACT: The objective of this research is to outline the liberation of indomethacin from different chitosan gels containing O/W microemulsion. The influence of surfactant, sodium lauryl sulfate, in two concentrations (0.5% and 0.75%, w/w) was determined in dissolution medium on the release of indomethacin, which was used as poor water-soluble model drug. Chitosan gels were prepared in four different concentrations of chitosan—1%, 1.5%, 2%, and 3% (w/w). Microemulsion enhanced the liberation of the indomethacin from chitosan gels into all dissolution mediums. Adding the surfactant into phosphate-buffered saline decreased the amount of liberated indomethacin from microemulsion, gel mixture, but increased the drug liberation from pure chitosan gels. It was detected that with the increased concentration of chitosan in the samples, the amount of indomethacin liberated ($p < 0.05$) also increased. A conclusion was drawn that the liberation of indomethacin from chitosan gels was influenced by increased pH of the samples. The high viscosity induced a higher release of indomethacin from 3% (w/w) chitosan hydrogel at pH 5.8 as compared with 3% (w/w) chitosan hydrogel at pH 3.8. The highest percentage of released indomethacin was determined when a mixture of microemulsion gel with higher chitosan content was used. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association *J Pharm Sci* 103:3977–3984, 2014

Keywords: drug delivery; gels; chitosan; microemulsion; dissolution; surfactants; permeation enhancers; semi-solids

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used as analgesics and for the treatment of local and systemic inflammatory pathologies. The limited efficacy of these agents, coupled with the strong predisposition to cause gastrointestinal (GIT)-associated adverse effects and nephrotoxicity via conventional routes prompted for exploitation of alternative drug delivery systems, for example, transdermal delivery.¹ The main drive behind topical application of NSAIDs is that blood concentrations are typically less than 1/20th of those found in orally used NSAIDs, minimizing the risk of serious harm.² Optimization of the systemic profile of indomethacin by controlled input of the drug via transdermal delivery has been shown to reduce GIT and central nervous system-related side effects.³

Approximately 40% of new chemical entities exhibit poor aqueous solubility and present major challenge to modern drug delivery systems because of poor absorption, poor bioavailability, and lack of dose proportionality.⁴ The selected drug for the study was indomethacin, which is an anti-inflammatory agent having low solubility. The preferred route of topical administration for indomethacin is either dermally or transdermally, for that reason it has to penetrate into the deeper skin layers or permeate the skin. Most topically administered drugs do not have the ability to penetrate the stratum corneum. In these cases, modulations of the skin penetration profiles of these drugs and skin barrier manipulations are necessary. A skin penetration enhancement can be achieved chemically, physically, or by use of appropriate formulations.⁵

By the *in vitro* assessment of dissolution properties of some low-solubility compounds, adequate dissolution cannot be obtained with aqueous solutions within physiologic pH ranges. It is optional for these solutions to utilize surfactant to enhance drug solubility. Commonly acceptable are ionic or nonionic surfactants, including sodium lauryl sulfate (SLS). A surfactant can be used as either a wetting agent or, when the critical micelle concentration (CMC) is reached, to solubilize the drug substance. The amount of surfactant needed for adequate drug solubility depends on the surfactant's CMC and the degree to which the compound partitions into the surfactant micelles. On the contrary, surfactant's CMC depends on the surfactant itself and the ionic strength of the dissolution medium.⁶

Sodium lauryl sulfate was used as a model anionic surface-active compound because its properties in aqueous solutions are well characterized.⁷ Various studies have reported the influence of surfactants on dissolution of pharmaceutical active ingredients.^{8–11} Surfactants are employed in dissolution studies because natural surfactants in the body aid in the dissolution and subsequent absorption of drugs with limited aqueous solubility.¹² Topical preparations are mostly used for the local effects on the site of their application by virtue of drug penetration into the underlying layers of skin or mucous membranes.¹³

The barrier for topical delivery is skin, which makes the drug delivery difficult. Taking this factor into consideration, microemulsions as the colloidal vehicle systems offer very good conditions for the fast and deep penetration of biologically active substances into the skin layer.^{14,15} Moreover, it has been reported that the ingredients of microemulsions may reduce the diffusion barrier of the stratum corneum and enhance the permeation of drug. Hence, it is promising for both transdermal and dermal delivery of drugs as an efficient route of drug administration. However, the low viscosity of microemulsion restrains its application in the pharmaceutical industry.^{16,17} The

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main advantage of microemulsions over the current formulations like topical gels and solutions used for treating pain or inflammation is high-solubilizing capacity for lipophilic drugs and enhanced topical drug availability.^{18,19} They can also influence a drug release from the formulation to enhance absorption or to lower toxicity.²⁰

Even though microemulsions offer several advantages for delivery, it is difficult to adjust the system for convenient topical application because of low viscosity. This problem can be overcome by using polymers such as chitosan as gelling agent.

Chitosans are linear cationic polysaccharides that are prepared by (partial) N-deacetylation of chitin, an abundant structural polysaccharide contained in crab and shrimp shells. Chitosans consist of (1→4)-linked 2-acetamido-2-deoxy- β -D-glucopyranose (GlcNAc) and 2-amino- β -D-glucopyranose units.²¹ Chitosan is regarded as such valuable natural biocompatible polymer because it is nontoxic, biodegradable, mucoadhesive, easily bioabsorbable, and also possesses gel-forming ability at low pH.²² Both the number of GlcNAc units (degree of acetylation) and the molecular weight of chitosans have been shown to influence the physical and biological properties of the polymer.²¹ At relatively low pH (<6.5), chitosan is positively charged and tends to be soluble in dilute aqueous solutions, but at higher pH it tends to lose its charge and may precipitate from solution because of deprotonation of the amino groups.⁷ Because of its polymeric cationic characteristics, chitosan can interact with negatively charged molecules or polymers.²³ Chitosan acts as a penetration enhancer by opening the tight epithelial junctions and hence is particularly exploited in protein and vaccine delivery.²⁴ Another important application of chitosans in industry is the development of drug delivery systems. The use of controlled-release systems has certain advantages as compared with conventional dosage forms, as they can minimize side effects, and prolong the efficacy of the drug. These dosage forms regulate the drug release rate and can reduce the frequency of administration of the drug, thus assuring better patient compliance. Pulsatile delivery systems based on chitosan have also been described, which are interesting with regard to adjusting drug release to physiological needs of the body, as in the case of hormone release. The potential of chitosan as a novel excipient, which might yet receive extensive application in pharmaceutical products, has been highlighted in several reports.²⁵

In our previous studies, we have investigated *in vivo* local anesthetic effects and acute toxicity of carbamate local anesthetics as carbisocaine, heptacaine, and pentacaine applied in W/O microemulsion vehicles.^{26–28} Later, we studied bicontinuous gel-like microemulsion dispersed systems as a vehicle for NSAIDs—indomethacin and diclofenac. Their permeation profiles through excised hairless rat skin in the *in vitro* experiment were determined.¹³ The effect of microemulsion on liberation of indomethacin from hydrophilic (carbopol) and lipophilic gel (aerosil in liquid paraffin) was also evaluated.²⁹

In this study, chitosan hydrogel formulations containing microemulsion as vehicles for indomethacin as drug were studied.

MATERIALS AND METHODS

Materials

Indomethacin [1-(p-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid] was obtained from Merck KGaA

(Darmstadt, Germany). Chitosan (medium molecular weight, MMW, Brookfield viscosity 200,000 cps), oil phase, and surfactant were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany), analytical-grade SLS was purchased from Sigma Chemical Company (St. Louis, Missouri). All other chemicals used were of analytical reagent grade. Purified water was used for the preparation of all samples.

Methods

Preparation of the Microemulsion Systems

The microemulsion composed of 24% (w/w) rosemary oil, 30% (w/w) water, and 46% (w/w) Tween80/IPA as surfactant–cosurfactant mixture (S/CoS) in ratio of 3:1 was prepared. The composition of the microemulsion was chosen according to preliminary trials. Rosemary oil and S/CoS mixture were mixed in the chosen concentrations, and then water was added dropwise with continuous stirring. The system was stored at room temperature in a tightly closed glass container until further use.

Preparation of Gels

Chitosan gels were prepared at 1%–3% (w/w) concentration in dilute lactic acid solution 1% (w/w). Indomethacin was incorporated into the formulations at 1% (w/w) concentration. The pH of each formulation was determined after 24 h hydration (pH meter, HI 991001; Hanna Instruments Ltd., SK). The mixture was stirred manually for 10 min and sonicated for 30 min.

Preparation of Microemulsion Chitosan Gel Samples

The appropriate amounts of indomethacin, microemulsion, and chitosan gel were weighed in the ratio 0.5:1:4. Indomethacin was added in a concentration of 1% (w/w). The mixture was mixed by using a magnetic stirrer (0.8 cm), until the samples were homogenized.

Dissolution Media

Preparation of phosphate buffer saline (PBS) pH 7.4 was prepared by mixing appropriate volumes of 1.6 mM KH_2PO_4 , 5.1 mM Na_2HPO_4 , and 154 mM NaCl. SLS was dissolved with the PBS (pH 7.4) in the concentration of 0.5% and 0.75% (w/w).

Rheological Characterization

Rheological experiments were performed to examine the viscous and elastic properties of the different formulations. Viscosity measurements of gels were performed on a controlled rate rotational viscometer (Viscotester VT 500, HAAKE, Berlin, Germany) at 20°C temperature.

Texture Analysis

A Texture Analyser TA.XT Plus (Stable Micro Systems Ltd., Surrey, UK) was used to determine the texture properties of the chitosan hydrogels. The gel formulation (60 g) was filled in a standard beaker (100 mL) and was kept in the ultrasonic water bath to remove air bubbles for 45 min. The disc was moved 1 mm from the bottom of the gel and rested for 30 s to relieve air bubbles under the disc. Starting point for the 35-mm disc was submerged (10 mm) and rested for 30 s. A disc (35 mm diameter) was pushed at a speed of 4 mm/s for a distance of 10 mm into the hydrogel and redrawn. Five replicate analyses were run with 30 s rest between every run at room temperature for each formulation. Data collection and calculation were performed using

Table 1. pH Values of Chitosan Hydrogels (Without Microemulsion) and Surface Tension Values of Dissolution Mediums with SLS at the End of 6 h of Release Through Nephrophan® (*n* = 5)

Type of Hydrogel and Concentration (% w/w)	pH Values of Chitosan Hydrogels	Initial Surface Tension of PBS with 0.5% SLS (γ , mN/m)	Final Surface Tension of PBS with 0.5% SLS (γ , mN/m)	Initial Surface Tension of PBS with 0.75% SLS (γ , mN/m)	Final Surface Tension of PBS with 0.75% SLS (γ , mN/m)
–	–	35.874 ± 0.099	–	35.312 ± 0.091	–
Chitosan, 1	3.30		35.914 ± 0.066		35,415 ± 0.058
Chitosan, 1.5	3.80		36.154 ± 0.106		35,865 ± 0.089
Chitosan, 2	4.02		36.553 ± 0.210		36,413 ± 0.143
Chitosan, 3	5.80		36.784 ± 0.247		36,442 ± 0.185

the Texture Exponent 32 (3.0.5.0) software package of the instrument. Gel parameters such as hardness, cohesiveness, and adhesiveness were determined from the resultant force–time plot. The maximum force does hereby present the hardness of the hydrogel formulation. Cohesiveness is defined as the work required to deform the hydrogel in the down movement of the probe. The second area shows the adhesiveness of the hydrogel to the probe.³⁰

Surface Tension Measurement

The surface tension of the selected mediums was determined by drop count method, using Traube's stalagmometer. The stalagmometer was filled with purified water above the upper mark. Using the screw pinch cork, the flow rate was adjusted to 10–15 drops/min. The number of drops of water was counted between the marks of the stalagmometer (n_1). Water was removed and the stalagmometer was filled with PBS (pH 7.4) containing SLS in concentration 0.5% or 0.75% (w/w) and number of drops was counted (n_2). All dissolution mediums with SLS were measured again after 6 h of liberation of a sample. The surface tensions of the dissolution mediums were determined using formula given below.

$$\text{Surfacetension}(\gamma_2) = n_1 \rho_2 \gamma_1 / n_2 \rho_1$$

where n_1 is number of water drops, n_2 is number of drops of sample, ρ_1 is density of water (0.99820 g/mL; 20°C), ρ_2 is density of sample, and γ_1 is surface tension of water (72.8 mN/m)

In Vitro Release of Drug

The release of indomethacin from different chitosan gels and their mixtures with microemulsion was determined by using Franz diffusion cells with dialysis cellulose membrane (Nephrophan®; VEB Filmfabrik, Wolfen, Germany). The artificial membrane was mounted between the receptor and donor compartments. The donor compartment was charged with 1.2 g of samples. The receptor compartment was filled with volume of PBS (pH 7.4) containing SLS in concentration 0.5% and 0.75% (w/w), which was maintained at 37 ± 0.5°C and stirred by magnetic bar at 200 ± 5 rpm. The available diffusion area of cell was 2.54 cm². The system was maintained throughout the experiment at 37°C. Five milliliter of medium was withdrawn at intervals of 15, 30, 45, 60, 90, 120, 180, and 360 min. The volume of each sample was replaced by the same volume of fresh buffer to maintain constant volume. Samples were analyzed for content of indomethacin spectrophotometrically at λ_{max} = 320 nm (UV/VIS spectrophotometer, Phillips PV 9652 UV/VIS, Phillips, Cambridge, Great Britain).

Statistical Analysis

Experimental results were expressed as mean ± SD (*n* = 6). Student's *t*-test was applied to control significant differences in drug release from different formulations. Differences were considered to be statistically significant at *p* < 0.05.

RESULTS AND DISCUSSION

Gels were prepared from chitosan (Table 1), after overlaying of lactic acid in different concentrations and readymade O/W microemulsion was used. Microemulsion shows the Z-average diameter of 21.60 nm and the polydispersity index 0.125 (Zetasizer Nano NS ZEN3600; Malvern Instruments Ltd, Worcestershire, UK).

The effect of microemulsion on the chitosan gels was evaluated by change of color, clarity, and viscosity. After addition of the microemulsion into the chitosan hydrogels, the mixture stayed opalescent and viscous.

Rheological measurements of chitosan gels show that they exhibit pseudoplastic flow in the 1%, 1.5%, and 2% concentration (Fig. 1). Gels (3%) of chitosan have slightly thixotropic behavior, which is more apparent in higher pH value (Figs. 2 and 3).

Texture profile analysis was used to investigate effects of pH of the formulation on the mechanical properties of 3% chitosan gels. The amount of lactic acid is expected to affect the pH of the formed hydrogel. The texture analysis can determine the changes of gel properties in relation to the changes in pH of the gel formulation. The chitosan hydrogel had rather stable texture properties at pH 5.8 as can be seen in Table 2. In 3% chitosan hydrogel at pH close to neutral, the texture forces (both force 1 and force 2) were found to be increasing. This is because of a higher viscosity of 3% chitosan hydrogel at higher pH. At a low pH, the measured forces (both force 1 and force 2) were found to be decreasing. The pH is affecting the swelling and properties of the polymer. Chitosan hydrogel has a high-ionization degree in acid medium, thus –NH₂ groups are in more protonated (–NH₃⁺) forms than chitosan hydrogel at pH close to neutral. Therefore, as the hydrogel is mainly in ionized form, it is able to bind anions of drug by electrostatic attraction. At low pH, the cohesiveness (Area 1) and the adhesiveness of chitosan gel (Area 2) were found to be decreasing. The increasing pH value of the chitosan formulation results in more coherent hydrogels as both gel cohesiveness and adhesiveness increased. The chitosan network appears to be stable at higher pH value. The high viscosity induced a higher release of indomethacin from 3% chitosan hydrogel at pH close to neutral than 3% chitosan hydrogel at pH 3.8.

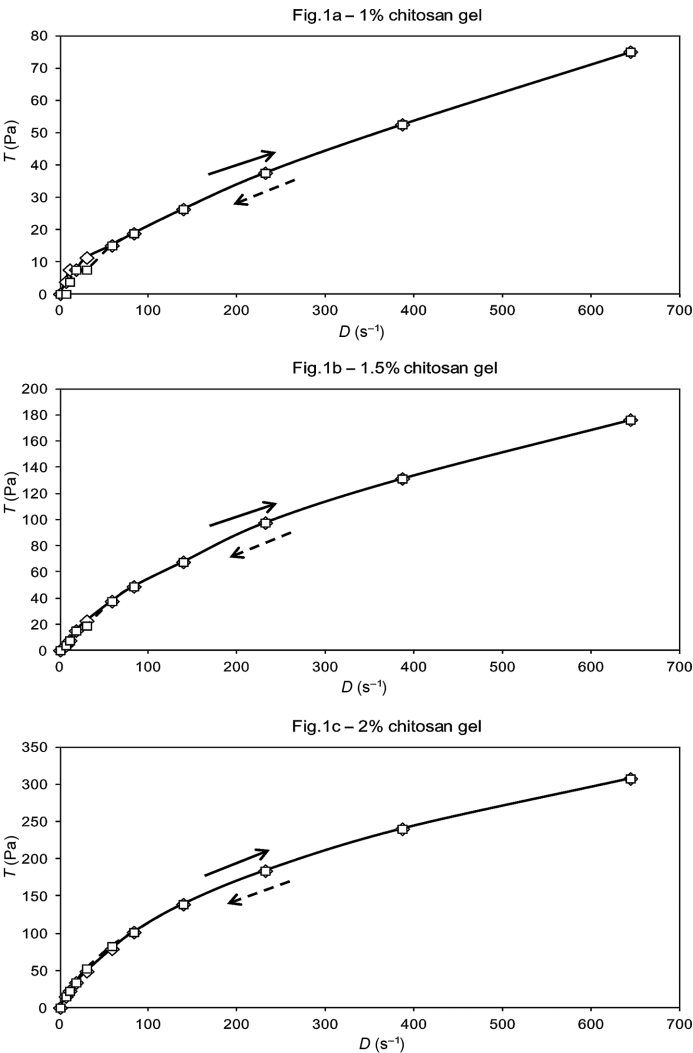


Figure 1. Rheogram of the chitosan gels (without microemulsion)—1% (a), 1.5% (b), and 2% (c).

Figure 4 shows the effect of concentration of chitosan on released amounts of indomethacin into different buffer solutions after 6 h. As evident from the figure, with increasing concentrations of chitosan, released amount of indomethacin rises. The highest amount of indomethacin was released from all prepared 3% chitosan gels (with or without microemulsion) in different buffer solutions.

Statistically significant differences were found ($p < 0.05$) between all drug release profiles from the gels in 3% concentration of chitosan in comparison to 1%, 1.5%, and 2% chitosan gels in all dissolution mediums used for liberation.

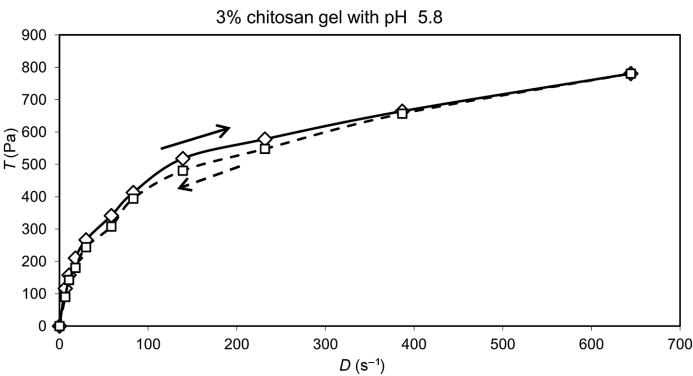


Figure 2. Rheogram of 3% chitosan gel at pH 5.8 (without microemulsion).

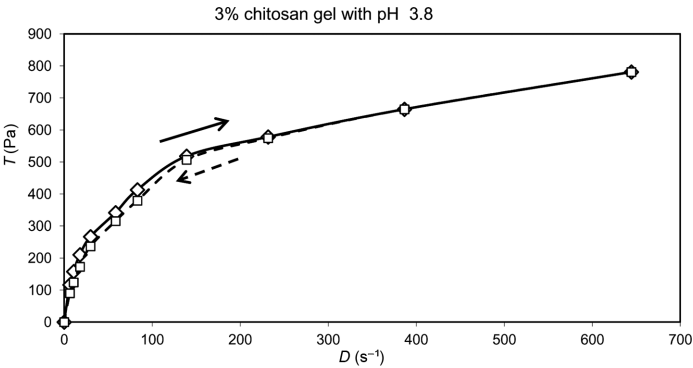


Figure 3. Rheogram of 3% chitosan gel at pH 3.8 (without microemulsion).

Drug release rates through the cellulose membranes of microemulsion formulations were compared with the release rates of the chitosan gels. It was observed that the presence of microemulsion caused the increased release of indomethacin from estimated samples (Fig. 4). Table 3 shows a significantly higher and faster drug release rates across the membrane for microemulsion–gel mixtures than for pure gels. Release of the drug through the membrane from microemulsion formulations was faster, even during the initial hours, than that from pure gels, which may be because of the fact that indomethacin dissolved better in the microemulsion, leading to higher concentration gradients toward the membrane. The high-solubilizing capacity of microemulsion enables to increase the solubility of indomethacin in PBS and the possibility of transmembrane delivery of a drug incorporated in microemulsion. Microemulsion provided higher and faster release rate than the gel, which may be related to the reduction in interfacial tension between the membrane and vehicle and perhaps because of the larger

Table 2. The Effect of pH on Texture Properties of Chitosan Hydrogels (Without Microemulsion) (3%, w/w) ($n = 5$)

Type of Hydrogel and Concentration (% w/w)	Force 1 \pm SD (g) (Maximum Compressing Force; Hardness)	Area 1 \pm SD (g*s) (Cohesiveness)	Force 2 \pm SD (g) (Minimum Retracting Force)	Area 2 \pm SD (g*s) (Adhesiveness)
Chitosan, 3 (pH 3.8)	49.440 \pm 0.114	104.702 \pm 0.311	–36.963 \pm 0.188	–73.099 \pm 0.870
Chitosan, 3; (pH 5.8)	150.780 \pm 1.036	274.666 \pm 1.543	–67.069 \pm 0.224	–87.173 \pm 1.645

Five replicate analyses were performed for each formulation, under the optimized conditions for that type of hydrogel.

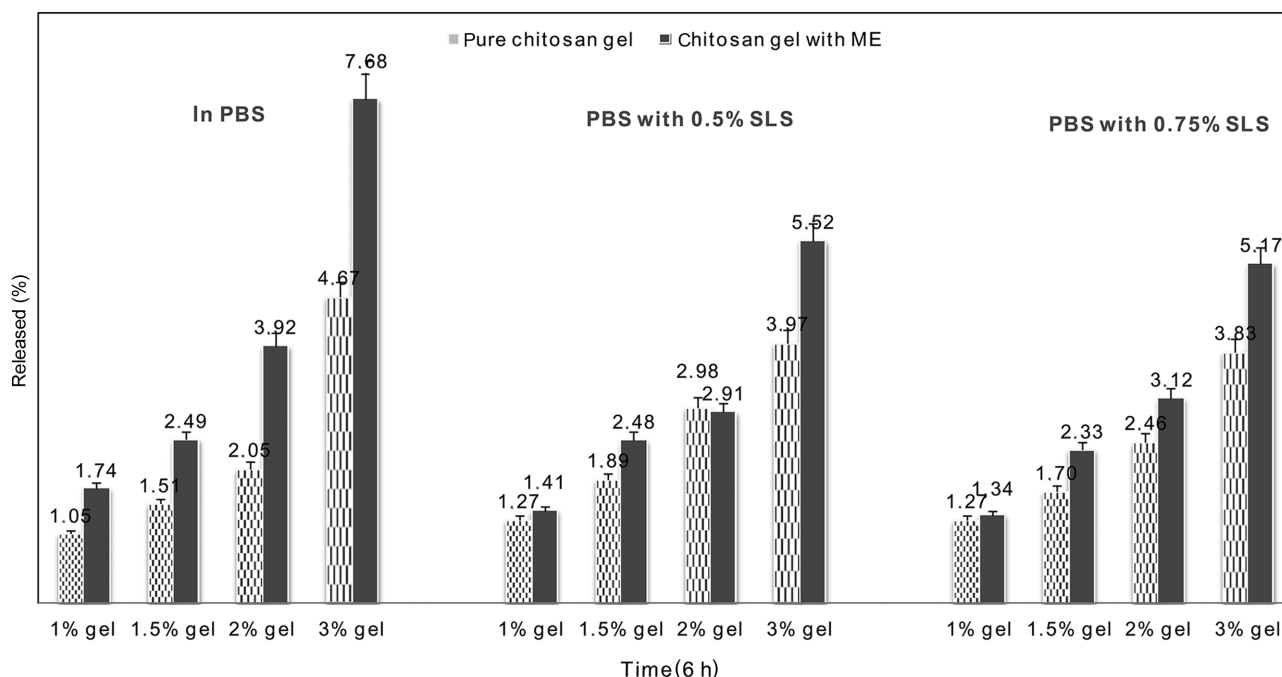


Figure 4. Released amount of indomethacin from chitosan gels and their mixtures with microemulsion in PBS and PBS with 0.5% and 0.75% (w/w) of SLS after 6 h. ME, microemulsion.

Table 3. Kinetic Parameters of Indomethacin Released from Various Chitosan Gels and Their Mixtures Containing Microemulsion

Dissolution Medium		Zero Order: $Q = K_0 t$		Higuchi: $Q = K_H t^{1/2}$		Zero Order: $Q = K_0 t$		Higuchi: $Q = K_H t^{1/2}$		
Gel	SLS (% w/w)	K_0 (% min ⁻¹)	$r^2/360$ min	K_H (% min ^{-1/2})	r^2	Gel*	K_0 (% min ⁻¹)	$r^2/360$ min	K_H (% min ^{-1/2})	r^2
G _{1%}	0	0.0026	0.9403	0.0347	0.6722	G _{1%} *	0.0039	0.8453	0.0477	0.5128
G _{1%}	0.5	0.0036	0.9526	0.0514	0.859	G _{1%} *	0.0034	0.9002	0.0455	0.6310
G _{1%}	0.75	0.0031	0.8694	0.0411	0.626	G _{1%} *	0.0034	0.9541	0.0454	0.6935
G _{1.5%}	0	0.0040	0.9727	0.0552	0.7676	G _{1.5%} *	0.0058	0.8996	0.0742	0.5832
G _{1.5%}	0.5	0.0047	0.9410	0.0641	0.6949	G _{1.5%} *	0.0058	0.8932	0.0735	0.5753
G _{1.5%}	0.75	0.0042	0.9322	0.0553	0.6402	G _{1.5%} *	0.0055	0.9059	0.0709	0.5997
G _{2%}	0	0.0053	0.9813	0.0719	0.7277	G _{2%} *	0.0093	0.9156	0.1188	0.5945
G _{2%}	0.5	0.0083	0.9417	0.1177	0.8392	G _{2%} *	0.0073	0.9575	0.0958	0.6702
G _{2%}	0.75	0.0062	0.9585	0.0813	0.6761	G _{2%} *	0.0077	0.9522	0.1007	0.6517
G _{3%}	0	0.0138	0.9644	0.1974	0.8942	G _{3%} *	0.0204	0.9913	0.2749	0.7405
G _{3%}	0.5	0.0105	0.9882	0.1425	0.7504	G _{3%} *	0.0154	0.9954	0.2135	0.8157
G _{3%}	0.75	0.0101	0.9870	0.1355	0.7362	G _{3%} *	0.0136	0.9827	0.1809	0.7147
G _{3%**}	0	0.0038	0.6965	0.0575	0.9868	G _{3%**}	0.0038	0.5840	0.0585	0.9769
G _{3%**}	0.5	0.0024	0.7123	0.0362	0.9552	G _{3%**}	0.0031	0.7980	0.0470	0.9517
G _{3%**}	0.75	0.0022	0.8856	0.0321	0.9479	G _{3%**}	0.0037	0.9333	0.0533	0.9248

Gel = Gel(% w/w, of chitosan); Gel* = Gel(% w/w, of chitosan) containing O/W microemulsion in 4:1 ratio; G_{3%**} = acidified gel; pH 3.8.

viscosity of the chitosan gel as compared with the microemulsion, which would have delayed the release of indomethacin from the pure gel.

As evident from the figure, the presence of the SLS (in concentration 0.5% and 0.75%) in PBS caused reduction in the amount of released indomethacin from samples containing microemulsion in comparison with the buffer solution without SLS. This difference may be because of the interaction of polymer and SLS from PBS, which creates the coats of solid gel on the surface of membranes in donor part, thereby slowing down the release process.

The higher concentration of SLS in dissolution medium (0.75%) slightly decreased the release of indomethacin, but the

difference between drug release profiles in PBS containing different concentrations of SLS was found to be statistically non-significant. Moreover, the presence of SLS in the dissolution medium also decreased the released amounts of indomethacin from pure 3% of chitosan gels (Fig. 4).

We found that the concentrations of SLS were decreased in PBS after 6 h of liberation. In other words, the surface tensions of all dissolution mediums containing SLS slightly increased after 6 h of liberation (Table 1). This means that the surfactant passed through the membrane into the donor part and interacted with our sample of chitosan. On this basis, we can say that the negative influence of SLS on the drug release is because of the complexation between SLS as an anionic

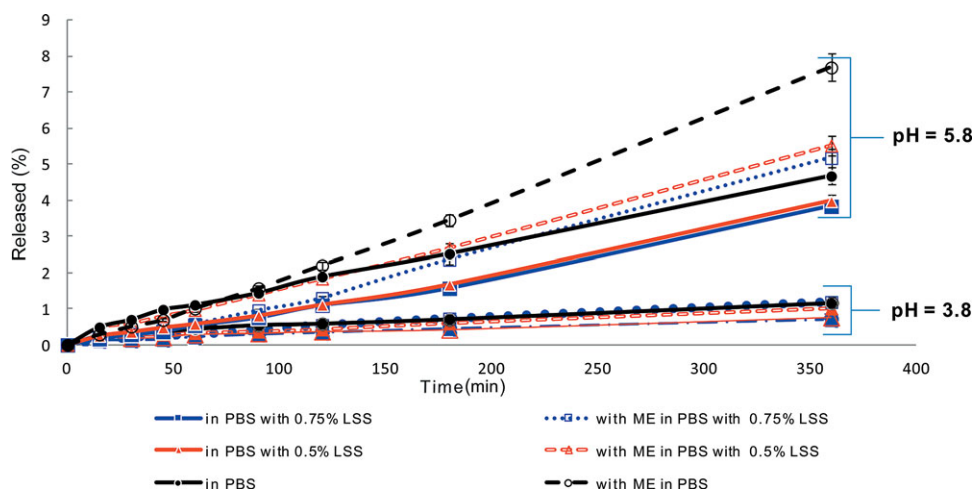


Figure 5. The effect of pH on indomethacin released profiles from 3% chitosan gels.

surfactant and chitosan as cationic polymer. SLS exhibits coulombic and noncoulombic interactions when kept in contact with polymeric surface. Their interactions are influenced mainly by coulombic forces between the ionic sites of surfactant and polymer. In our experiment, the formation of coat of solid gel on the surface of membranes in donor part after 6 h of release in all mediums with SLS was also observed. In the case of samples with microemulsion, the higher amounts of drug were released because of the fact that microemulsion provided higher release rate from these samples. In the presence of microemulsion, a lower viscosity of these samples was observed what caused that the coats of solid gel on the surface of membranes in donor part were thinner after 6 h of release.

In the case of 1%, 1.5%, and 2% chitosan gels without microemulsion, indomethacin was released in higher amount into the PBS containing SLS in comparison with pure PBS. The formation of solid coat of solid gel on the surface of membranes in donor part after 6 h of drug liberation was also observed, but the addition of SLS in PBS could probably enhance the dissolution rates of indomethacin by increasing contact between the drug and the dissolution medium. Further rise in the concentration of SLS to 0.75% in dissolution medium decreased the release of indomethacin. The CMC of SLS is 8.0×10^{-3} mol/L, that is, 0.23% at 25°C. Used concentration of SLS was more than CMC^{8,31} and decreased by the presence of inorganic salts in the dissolution medium. The higher ionic strength at higher concentration of SLS (0.75%) possibly affects the drug release by complexation with ionized amino groups of chitosan ($p < 0.05$). The formation of coat of solid gel on the surface of membrane was probably faster at a higher concentration of SLS and this could reduce the rate of drug release.

In this case, the complex forms layer on the permeation membrane; depending on the concentration of chitosan and SLS, this can modify the permeation by decreasing the amount of drug released.

The formation of these complexes may be particularly useful for the encapsulation or controlled release of certain components⁷ and may provide the required physicochemical properties for the design of specific drug delivery systems.³²

As evident from Figure 4, the highest amounts of indomethacin were released from all 3% chitosan gels with or without microemulsion. Varshosaz et al.³³ also published that

the release data reveal that increasing the concentration and molecular weight of chitosan increase the lidocaine release rate that is not in accordance with viscosity results. In other words, the higher the shearing rates of the gel, the faster the release of lidocaine. Senel et al.³⁴ reported an increase in chlorhexidine gluconate release rate by increasing the chitosan concentration.

In our work, chitosan inhibited the release of indomethacin from chitosan gels with low content of chitosan at low pH, possibly because of the formation of an ionic complex between the amino group of chitosan and carboxyl group of indomethacin. There was higher amount of released indomethacin from 3% chitosan samples, which is probably attributable to increased pH (5.8) and because of higher solubility of indomethacin and only partial ionization of amino groups (40% chitosan amino groups are protonated at pH 6.0).³⁵ Figure 5 shows the effect of pH on released profiles of indomethacin from 3% chitosan samples with pH 3.8 and 5.8 and their mixtures with microemulsion at pH 7.4 phosphate buffer and PBS with 0.5% and 0.75% content of SLS. From Figure 5, it can be seen that more of the indomethacin was released from the weakly acidic chitosan samples (5.8) in which the amino groups of chitosan are not completely protonized than chitosan samples at the pH 3.8, in which chitosan should be totally protonated. Chitosan carries primary amine functional groups and therefore its charge and solubility are pH dependent. Changes in pH have a clear influence on the release of the drug.

Polymer–drug interaction plays an important role in the release behavior of the drug. Hence, polymers interacting with drugs could be investigated to modulate the release profile of the drug. The regression data for two kinetic models—zero order and Higuchi—indicate that all samples containing not acidified gels follow zero-order kinetics. The amount of indomethacin released from 3% gel formulations with lower pH—acidified ($G_{3\%}^{**}$) studied here showed that the data fitted better in the Higuchi model as indicated by higher r^2 values. The plot of the cumulative indomethacin permeation through the membrane versus square root of time was almost linear, thus indicating diffusion controlled-release kinetics. In other words, the release of indomethacin from 3% acidified gel formulations is diffusion rate limited. The acidification of chitosan (pH 3.8) decreased drug release rate constants as compared with chitosan (pH 5.8) without change of pH. The principal mechanism of such

interactions is the formation of hydrogen bonds involving amino group of chitosan and the carboxyl group of indomethacin. The drug release rate constants of the test formulations are presented in Table 3.

These results indicate that chitosan gels could be convenient for controlled-release delivery systems of different drugs whenever pH-sensitive mechanics might be useful.

CONCLUSIONS

Microemulsion enhanced the liberation of the indomethacin from chitosan gels into all dissolution mediums. Adding of the surfactant into PBS decreased the amount of liberated indomethacin from microemulsion–gel mixtures but increased the drug liberation from pure chitosan gels with lower concentration of chitosan. With the increased concentration of chitosan in the samples, the released amount of indomethacin was also increased. Higher pH of the samples was decisive for the liberation of indomethacin from chitosan gels. The increasing of pH value of the chitosan formulation results in more coherent hydrogels. Both the gels cohesiveness and adhesiveness increased. It appears that higher pH value stabilizes the chitosan network. The high viscosity induced a higher release of indomethacin from 3% chitosan hydrogel at pH 5.8 as opposed to 3% chitosan hydrogel at pH 3.8. The highest percentage of released indomethacin was determined when a mixture of microemulsion gel with higher chitosan content was used. On the basis of our previous study,²⁹ it can be concluded that this prepared chitosan gel with microemulsion is more suitable for permeation of indomethacin than commercial Indobene Gel®. Chitosan gels could be convenient for the controlled release of drugs.

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